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Peptides and their use for the treatment of hiv infections

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PEPTIDES AND THEIR USE FOR THE TREATMENT OF HIV INFECTIONS

The present invention relates to peptides which exhibit inhibitory activity on the infection of human cells by human immunodeficiency virus (HIV).

In the last years, intensive research for therapeutics with activity against infection by HIV was performed. Several medicaments were developed and tested, which delay and suppress the outbreak of AIDS and lower the level of the HIV in blood. In the US the life-span of HIV-infected patients after the outbreak of AIDS was raised from 11 month in 1984 to 46 month in 1997.

In the search for therapeutics various strategies were applied, which lead to several classes of medicaments such as the protease-blockers inhibiting a protease, which the virus requires for replication, and medicaments inhibiting the viral reverse transcriptase, which is essential for the replication of retroviruses. A group of possible active agents developed only recently are fusion inhibitors, which shall prevent the entry of the virus into cells. It was also shown that the provision of interleukin-2 in combination with other active agents could increase the strength of the immune response.

A further agent with biological activity against HIV was recently described in WO01/34640. Disclosed is a peptide of 20 amino acids named VIRIP (virus inhibiting peptide), which was isolated from human hemofiltrate and found to inhibit the infection of human cells by HIV.

Despite those efforts and different available medication, the problem remains unsolved that there is still no cure against AIDS, because the known therapeutics, though capable of significantly lowering the level of HIV in the body and of HIV-infected blood cells, do not remove the virus entirely. A special drawback is, that the HIV is especially prone to mutations, which often result in the development of resistance against certain therapeutics. In general, the known therapeutics are only sufficiently effective if they are administered in combination with other therapeutics. Such combined therapies

at present extend the lifespan of the average patient without providing a cure, and are generally accompanied by severe side effects and frequently do not allow the patient to lead a „normal" life.

There is a great medical need to to provide new therapeutics and improved therapeutics, which will lead to improved therapies, less side effects, and significant extension of the life expectancy of those infected by HIV, before or after the outbreak of AIDS.

The present invention faces the problem to provide new therapeutics, which will overcome the problems as described above, and will allow an efficient therapy or will contibute to an efficient combination therapy.

Surprisingly, the problem is solved by peptides having the amino acid sequence

Z_1-LEAIP-X_3-SIP-X_1-X_4-V-X_2-FNKPFVF-Z_2,

wherein

X_1 is a D-proline or L-proline;

X_2 is an amino acid with a hydrophobic or an aromatic side chain or lysine;

X_3 and X_4 are cysteines, or X_3 is methionine and X_4 is glutamic acid;

Z_1 is NH_2 or a sequence of 1 to 10 amino acid residues;

Z_2 is COOH or a sequence of 1 to 10 amino acid residues;

and peptides which are fragments and/or covalently linked oligomers and/or derivatives, especially amidated, acylated, sulfated, pegylated, phosphorylated and/or glycosylated derivatives, and mutants thereof,

with biological activity against infection by HIV,

with the proviso that at least one of the following is true:

X_1 is D-proline or

X_2 is not lysine or

X_3 and X_4 are cysteine.

Specific embodiments of peptides of the invention are those according to any of claims 2 to 5.

The peptides of the invention are related to the hemofiltrate-derived peptide VIRIP as disclosed and described in WO01/34640, which has biological activity in preventing infection by HIV.

The peptides of the invention are based on an amino acid sequence of 20 amino acids, with possible extensions at both ends according to Z_1 and Z_2. The amino acid numbering used herein always corresponds to the amino acids 1 to 20 of the basic sequence, irrespective of a possible N-terminal extension due to a residue Z_1, such that amino acid position 1 corresponds to leucine and amino acid position 20 to phenylalanine. The common amino acid one and three letter codes are used. The small letter "p" stands for D-proline.

The term "hydrophobic amino acid" as used herein is readily understood by the skilled person. In particular, it refers to any of the amino acids glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan, and non-endogenous hydrophobic amino acids.

The term "aromatic amino acid" as used herein is readily understood by the skilled person. In particular it refers to any of the amino acids phenylalanine, tyrosine, tryptophane, and non-endogenous aromatic amino acids.

The term "mutants" is readily understood by the skilled person. In particular, it refers to sequence variants, in which one or more of the amino acids as disclosed are changed. Mutants of the invention preferably vary from a peptide of claim 1 by one, two, three or four amino acids. In a preferred embodiment, the mutations are conservative, such that the properties of the side chain are not altered substantially. Mutants also include sequence variants, wherein one or more amino acids are deleted from the sequence or inserted into the sequence.

The term "fragments" is readily understood by the skilled person. In particular it refers to sequence variants in which the sequence is truncated at the N- or C-terminus. In preferred embodiments, the peptides lack up to 2, 4 or 6 amino acids at the N- and/or C- terminus.

Surprisingly, it was found that by few specific variations in the amino acid sequence of VIRIP, the activity against HIV is increased significantly. The most significant increase in activity is observed, when the L-proline at position 10 is substituted by a D-proline, and/or two cysteines are introduced at amino acid positions 6 and 11, and/or when the positively charged lysine at position 13 is exchanged against an amino acid with a hydrophobic or aromatic side chain. The three substitutions may be combined in peptides of the invention. It is believed that the activity when compared to wild-type VIRIP is increased due to a change in structure. Cysteine bridges are known to alter the structure of a peptide significantly, as well as the introduction of a D-proline, which imposes rigid restrictions on the flexibility of the peptide chain. Furthermore, the exchange of a lysine against an uncharged hydrophobic or aromatic amino acid will alter the structure, because a possible interaction of the positively charged lysine side chain with the negatively charged amino acids at positions 2 and 11, or with a negatively charged portion of a receptor molecule is disrupted.

Peptides according to the invention can be chemically synthesized or produced by recombinant expression. The chemical synthesis can be carried out on a solid support using solid-phase technologies or in solution phase, both being standard methods known to the skilled person. Peptides according to the invention can also be synthesized by the ligation of two or more side chain-protected or side chain-unprotected fragments, standard methods known to the skilled person. The solid-phase synthesis of peptides according to the invention or its fragments can be carried out using the Fmoc/tBu- or Boc/Bzl-protection pattern of amino acids. Purification of synthetic peptides is achieved by chromatographic methods such as reverse-phase, ion exchange or size-exclusion.

The introduction of a disulfide bond into peptides of the invention may be achieved by oxidative methods known to the skilled person (Pennington and Dunn, Editors, Peptide Synthesis protocols, Humana Press, Totowa, 1994; Chan and White, Editors, Fmoc Solid Phase Peptide Synthesis: A Practical Approach, Oxford University Press, Oxford, 2000). Disulfides of peptides of the invention may be generated from reduced precursor peptides containing one or two cysteine residues obtained from solid-phase or solution synthesis by oxidative treatment. As oxidizing agents oxygen, dimethylsulfoxide, iron(III) salts, iodine, or others may be used. Disulfides of peptides of the invention may alternatively be introduced into the peptides from precursors containing protective groups at the corresponding cysteine residues. As protective groups acetamidomethyl, tert-butyl, S-tert-butyl or others may be used. Cleavage of protective groups and intra-chain disulfide bond formation may be carried out using agents such as iodine, phosphines, or others.

An N-alkylated amide bond between the leucine at amino acid position 1 and the glutamic acid at amino acid position 2 is obtained by using an N-alkylated glutamic acid derivative, which is used in the solid-phase or solution synthesis of peptides of the invention as a N-terminally Fmoc- or Boc-protected amino acid. N-alkyl may also be introduced after the chain assembly to a glutamic acid residue contained in precursors of peptides of the invention by methods known to the skilled person (Greene and Wuts, Protective Groups in Organic Chemistry, John Wiley & Sons, New York, 1991).

The invention also relates to nucleic acids coding for peptides of the invention. Preferred nucleic acids are DNA and RNA, especially cDNA and mRNA.

Subject of the invention are also antibodies specifically binding to peptides of the invention. The term "specifically" is readily understood by the skilled person. In particular, it means that the antibodies do not bind or do essentially not bind related peptides like VIRIP which are not peptides of the invention. A person skilled in the art obtains antibodies against peptides of the invention by routine methods, and will select specific antibodies of the invention by known screening methods.

The invention also relates to a medicament containing the peptides, nucleic acids or antibodies of the invention. The medicament is preferably provided in galenic formulations for oral, intravenous, intramuscular, intracutaneous, subcutaneous, intrathecal administration, or as an aerosol for transpulmonary administration.

In a preferred embodiment, the medicament comprises at least one further therapeutic agent. Said at least one further therapeutic agent can be a viral protease inhibitor, a reverse transcriptase inhibitor, a fusion inhibitor, a cytokine, a cytokine inhibitor, a glycosylation inhibitor or a viral mRNA inhibitor. Preferably, such inhibitors are directed against HIV. Such combined therapeutics are highly relevant in the treatment of AIDS. The peptides, nucleic acids and antibodies of the invention are preferably used in manufacturing of a medicament for the treatment of HIV infections. This comprises all known strains of the retrovirus HIV (human immunodeficiency virus), especially the most common HIV-1 and HIV-2. HIV-1 is associated with the outbreak of AIDS.

The invention also relates to a diagnostic agent containing peptides, nucleic acids or antibodies of the invention. The diagnostic agent may be used for assay systems for testing isolated plasma, serum, tissue, urine and cerebrospinal fluid levels for HIV infections.

Examples

Example 1: Chemical synthesis of VIRIP peptides

The peptides according to the invention were chemically synthesized utilizing the principle of solid-phase peptide synthesis and the Fmoc or Boc protective group strategy (Atherton and Sheppard, 1989, Solid Phase Peptide Synthesis, IRL Press; Merrifield, 1986, Solid phase synthesis, Science 232, 341-347), but can also be synthesized with solution phase synthesis or by coupling protected or unprotected fragments of the peptides according to the invention.

As an example, the synthesis of the peptide LEAIPMSIPpEFLFNKPFVF is described using fluorenylmethoxycarbonyl (Fmoc)-protected amino acids on an

automated peptide synthesizer 433A (Applied Biosystems). The synthesis was performed using a preloaded Fmoc-Phe-Wang resin with a loading capacity of 1 mmol/g resin with standard HBTU [(2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate]/HOBt (1-hydroxybenzotriazol) activation with capping cycles using acetic anhydride in N-methylpyrrolidinone (NMP) at a scale of 0.2 mmol. The side chains of the amino acid building blocks used were protected as follows: Glu(OtBu), Ser(tBu), Lys(Boc), Asn(Trt). Acylation steps for peptide chain assembly were carried out for 15-60 min, and Fmoc groups were deprotected with piperidine in NMP after each acylation. After deprotection of the leucine residue at position 1, the resulting protected peptidyl resin was washed with NMP, 2-propanol and dichloromethane and then dried. The dry resin was treated at room temperature with a fresh mixture of trifluoracetic acid/ethanedithiole/water (94:3:3, vol/vol/vol, 40 ml/g resin) for 2-4.5 h. The mixture was filtrated into ice-cold tert-butylmethylether (TBME) to facilitate precipitation of the peptide. The resulting precipitate was separated by centrifugation, washed with TBME and dried under vacuum. The crude peptide was dissolved in diluted acetic acid and loaded onto a preparative Vydac C18 column (47x300 mm, 15-20 µm, flow rate 40 ml/min; solvent A, 0.07 volume % TFA; solvent B, 0.07 volume % TFA in acetonitrile/H_2O 80:20 (volume %); UV detection at 215 nm; with the following gradient: 45-70 volume % B in 50 min. The fractions containing the desired pure peptide, as detected by mass spectrometry (API 100, Perkin Elmer) and analytical C18 HPLC or, alternatively, capillary zone electrophesis, were pooled and dried by lyophilization. The lyophilized peptide was used for analysis of purity and molecular weight by analytical C18 HPLC (Figure 1), capillary zone electrophoresis, and mass spectrometry (Figure 2). The yield of the peptide LEAIPMSIPpEFLFNKPFVF was 138 mg. The process for synthesis of the peptides according to the invention was adapted to larger scales ranging from 0.5 to 20 mmol yielding purified VIRIP peptides in amounts between 1 g and 5 g.

Peptides according to the invention having intramolecular disulfide bonds were treated with air at pH 7.5-8.5, with or without dimethylsulfoxide to facilitate cysteine.

Using this general synthetic approach, the following peptides, among others, were synthesized, purified by chromatographic methods to a degree of minimum 93% and analysed:

Peptide	Yield [mg]	mw (calculated)	mw (mass spectrometry)
VIR-121	109	2246.7	2246.5
VIR-161	34	2190.6	2190.3
VIR-162	56	2119.6	2119.0
VIR-163	98	2232.7	2232.8
VIR-164	35	2266.7	2266.5
VIR-165	72	2238.7	2238.0
VIR-166	37	2361.4	2362.3
VIR-170	50	2265.7	2267.0
VIR-175	105	2279.7	2279.5
VIR-182	56	2217.6	2217.2
VIR-184	82	2260.7	2260.4
VIR-190	71	2175.6	2175.2
VIR-191	25	2231.7	2231.8
VIR-192	53	2265.7	2265.0
VIR-193	138	2294.7	2295.0
VIR-197	50	2322.7	2322.3
VIR-199	138	2336.8	2336.5
VIR-229	58	2228.6	2228.3
VIR-234	78	2216.6	2217.0
VIR-243	34	2312.7	2312.7
VIR-252	142	2290.7	2290.3
VIR-255	56	2303.7	2303.5

VIR-257	151	2329.0	2328.2
VIR-258	50	2345.0	2344.4
VIR-259	110	2312.9	2312.4
VIR-260	162	2324.0	2323.4
VIR-261	79	2371.0	2370.3
VIR-262	61	2234.6	2334.3
VIR-263	147	2334.6	2334.3
VIR-264	102	2379.1	2378.5
VIR-265	118	2329.0	2330.0
VIR-266	175	2361.8	2361.2
VIR-268	123	2308.5	2308.3
VIR-269	46	2301.0	2300.3
VIRIP	265	2303.8	2303.6

Example 2: Cytotoxicity of VIRIP-peptides on human cells

The cytotoxicity of peptides of the invention was tested by evaluating the viability of human monocytic THP-1 cells. Cytotoxic effects of the peptides were tested by their influence on metabolic activity by means of the WST-1 assay (Roche Diagnostics, Germany). THP-1 cells were incubated with test peptides in a 96-well plate (approx. 25,000 cells per well) for 24 hours in RPMI-1640 medium containing 25 mM L-glutamine and 10 volume % fetal calf serum at 37 °C in an atmosphere with 5 volume % CO_2. Ten µl of a WST-1 solution was added to each cavity, and incubation of THP-1 cells was allowed for 2 further hours at corresponding conditions. Metabolically active THP-1 cells reduce WST-1, a light red tetrazolium salt, yielding a soluble yellow formazan salt. The amount of reduced WST-1 correlates directly to the number of living cells, and is measured photometrically at a wavelength of λ=450 nm using a microtiter plate reader (reference wavelength is 630 nm). As a positive control, the known cytotoxic substance cycloheximide was used at a concentration of 50 µg/ml; the cytotoxicity of cycloheximide was set to 100%. As another positive control the peptide MBI-28, a highly cytotoxic peptide

known to the skilled person, was used with a maximum concentration of 300 µg/mL. As a negative control, cultured THP-1 cells not treated with the peptides of the invention or a positive control were used. The cytotoxicity of VIRIP peptides was calculated using the formula

$$\text{Viability [\%]} = [A_{450\,nm}\ (\text{peptide}) - A_{450\,nm}\ (\text{cycloheximide})] / [A_{450\,nm}\ (\text{negative control}) - A_{450\,nm}\ (\text{cycloheximide})] * 100$$

and was correlated to the averaged viability of untreated THP-1 cells. . The experiments were carried out at concentrations of peptides according to the invention of 30 µg/mL, 100 µg/mL, 300 µg/mL and 1000 µg/mL. The peptides VIR-161, VIR-162, VIR-163, VIR-164, VIR-165, VIR-166, VIR-170, VIR-175, VIR-182, VIR-184, VIR-190, VIR-191, VIR-192, VIR-193, VIR-197, VIR-199, VIR-229, VIR-234, VIR-243, VIR-252, VIR-255, VIR-257, VIR-258, VIR-259, VIR-260, VIR-261, VIR-262, VIR-263, VIR-264, VIR-265, VIR-266, VIR-268, VIR-269, VIR-161, VIR-161, VIR-161, and VIR-161 were tested. These peptides did not exhibit a cytotoxic effect on monocytic THP-1 cells compared to the positive controls cycloheximid and MBI-28.

Example 3: Inhibition of the HIV infection by VIRIP peptides

P4-CCR5 indicator cells (Charneau et al., 1994, HIV-1 reverse transcription. A termination step at the center of the genome. Journal of Molecular Biology 241, 651-662) expressing the primary CD4 receptor and both major HIV-1 entry cofactors CXCR4 and CCR5, were used to evaluate whether peptides according to the invention are potent inhibitors of HIV-1 infection. These cells contain the ß-galactosidase reporter gene under the control of the HIV-1 promoter. Thus, activation of the ß-galactosidase reporter gene allows to measure the efficiency of HIV-1 infection and thus to quantitate the potency of HIV-1 inhibitors (Detheux et al., 2000; Natural proteolytic processing of hemofiltrate CC chemokine 1 generates a potent CC chemokine receptor

(CCR)1 and CCR5 agonist with anti-HIV properties. Journal of Experimental Medicine 192, 1501-1508; Münch et al., 2002; Hemofiltrate CC chemokine 1[9-74] causes effective internalization of CCR5 and is a potent inhibitor of R5-tropic HIV-1 strains in primary T-cells and macrophages. Antimicrobial Agents and Chemotherapy 46, 982-990).

To perform the infection assay, P4-CCR5 cells (Charneau et al., 1994) were kept in RPMI 1640 medium supplemented with 10 volume % FCS. This cell line coexpresses CD4 and both HIV-1 coreceptors CCR5 and CXCR4 and contains the ß-galactosidase gene under the control of the HIV-1 promoter. Virus stocks were generated by the calcium coprecipitation method as described (Detheux et al., 2000), and the p24 antigen levels were quantitated with an HIV p24 ELISA kit obtained through the NIH AIDS Reagent Program. Cells were seeded in flat-bottomed 96-well dishes, cultured overnight, and incubated with the different doses of peptide for 2 h before infection with virus containing 1 ng of p24 antigen in a total volume of 50 ml of medium. After overnight incubation, cells were washed twice and cultivated in fresh culture medium without inhibitory peptide. Three days after infection the cells were lysed, and infectivity was quantitated using the Galacto-Light Plus™ chemiluminescence reporter assay kit (Tropix, Bedford, MA) as recommended by the manufacturer. All infections were performed in quintuplicate.

The results of this assay demonstrate that peptides according to the invention have greatly enhanced anti-HIV-1 activity as compared to VIRIP. Peptides of the invention inhibited the infection by the X4-tropic HIV-1 NL4-3 and the HIV-1 NL4-3 DTV (Rimsky et al., 1998; Determinants of human immunodeficiency virus type 1 resistance to gp41-derived inhibitory peptides. Journal of Virology 72, 986-993) molecular clones with more than 10-fold up to more than 100-fold higher efficiency than the original VIRIP. Peptides of the invention were also active against infection by the R5-tropic HIV-1 YU-2 molecular clone. These data demonstrate that the specific modifications of VIRIP greatly enhance the anti-HIV-1 potency of peptides according to the invention. Below, the IC_{50} values of peptides of the invention obtained from the described infection assay are provided.

Peptide	IC_{50} [µM] HIV-1 NL4-3	IC_{50} [µM] HIV-1 NL4-3 DTV
VIR-121	0.37	1.79
VIR-161	0.55	0.57
VIR-162	0.66	0.95
VIR-163	0.76	0.29
VIR-164	0.34	0.37
VIR-165	0.27	0.14
VIR-166	0.27	0.33
VIR-170	1.52	2.00
VIR-175	0.70	0.30
VIR-182	2.25	2.97
VIR-184	1.99	5.39
VIR-190	1.84	3.11
VIR-191	1.79	0.56
VIR-192	1.54	1.21
VIR-193	1.74	1.38
VIR-197	1.27	1.44
VIR-199	2.14	1.65
VIR-229	1.28	2.26
VIR-234	0.74	6.41
VIR-243	2.16	1.98
VIR-252	1.75	1.87
VIR-255	0.65	1.23
VIR-257	0.86	0.66
VIR-258	0.64	0.62
VIR-259	0.86	1.03
VIR-260	2.15	2.38
VIR-261	0.67	0.52

VIR-262	0.94	0.58
VIR-263	0.77	0.33
VIR-264	0.59	0.70
VIR-265	1.29	2.21
VIR-266	0.59	0.83
VIR-268	1.73	1.48
VIR-269	2.61	0.90
VIRIP	15.00	22.20

Example 4: Toxicity of VIRIP peptides in mice

Acute toxicity was evaluated with VIR-121 (LEAIPMSIPpEVAFNKPFVF) after a single intravenous injection into the tail vein of SCID-C.B 17-mice. A dose of 927 mg VIR-121 dissolved in 13.6 ml 0.9 volume % sodium chloride solution per kg body weight (equivalent to 20.4 mg or 272 µL per mouse) was applied. Injection speed was dose within 15 seconds. Three animals were treated with the test substance, and the animals were observed at time points of 5, 15, 30 min, and 1, 3, 6, and 24 hours after administration of the sample into the tail vein. As a control, 3 mice were each treated with a corresponding volume of vehicle (0.9 volume % NaCl). After 24 hours, the animals were sacrificed, dissected and inspected macroscopically. During and after application until the end of the observation period of 24 hours for all animals treated with VIR-121 no signs of reduced or increased motility, dyspnea, ataxia, nor a reduced or increased muscle tone were observed. No changes of behaviour was observed, and behaviour was comparable to that of the control animals. No findings were obtained from macroscopic necropsy compared to the control group.

Figure legends

Figure 1

C18 HPLC trace of purified VIR-199 (sequence: LEAIPMSIPpEFLFNKPFVF). Conditions: Vydac C18 (4.6 × 250 mm, 300 Å, 5 µm, flow rate: 0.8 ml/min,

gradient: 10-70 volume % B in 30 min, buffer A: 0.07 volume % TFA, buffer B: 0.05 volume % TFA, 80 volume % acetonitrile).

Figure 2

Electrospray-ionization mass spectrum (ESI-MS) of purified VIR-199 (sequence: LEAIPMSIPpEFLFNKPFVF). The mass spectrum was recorded using a Sciex API 100 mass spectrometer.

Abbreviations:

tBu:	tert-butyl
Boc:	tert-butyloxycarbonyl
Trt:	trityl
TFA:	trifluoroacetc acid
HPLC:	high performance liquid chromatography,
MALDI-TOF:	matrix-assisted laser desorption/ionization-time-of-flight
Tic:	1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid
Oic:	octahydroindolyl-2-carboxylic acid

Claims

1. Peptides having the amino acid sequence

Z_1-LEAIP-X_3-SIP-X_1-X_4-V-X_2-FNKPFVF-Z_2,

wherein

X_1 is a D-proline or L-proline;

X_2 is an amino acid with a hydrophobic or an aromatic side chain or lysine;

X_3 and X_4 are cysteines, or X_3 is methionine and X_4 is glutamic acid;

Z_1 is NH_2 or a sequence of 1 to 10 amino acid residues;

Z_2 is COOH or a sequence of 1 to 10 amino acid residues;

and peptides which are fragments and/or covalently linked oligomers and/or derivatives, especially amidated, acylated, sulfated, pegylated, phosphorylated and/or glycosylated derivatives, and mutants thereof,

with biological activity against infection by HIV,

with the proviso that at least one of the following is true:

X_1 is D-proline or

X_2 is not lysine or

X_3 and X_4 are cysteine.

2. Peptides according to claim 1, which comprise one or more of the following amino acid substitutions:

X_3 is isoleucine and X_4 is glutamic acid;

the alanine at amino acid position 3 is substituted by aspartic acid or lysine

the serine at amino acid position 7 is substituted by glycine;

the valine at amino acid position 12 is substituted by leucine, isoleucine or an amino acid with an aromatic side chain, preferably phenylalanine, 1-naphtylalanine, p-fluorophenylalanine or 3,3-diphenylalanine;

the glutamine at amino acid position 15 is substituted by glycine or alanine;

the proline at amino acid position 17 is substituted by aspartic acid or L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (L-Tic) or octahydroindolyl-2-carboxylic acid (Oic).

3. Peptides according to claim 1 or 2, wherein the cysteine residues at positions 6 and 11 are connected by an intramolecular disulfide bond.

4. Peptides according to any of the claims 1 to 3, wherein the leucine residue at amino acid position 1 and the glutamic acid at amino acid position 2 are covalently linked by an N-alkylated amide bond or by an ester bond or by a reduced peptide bond or by a retro-inverso peptide bond or by an N-alkylated retro-inverso peptide bond.

5. Peptides according to any of the claims 1 to 4 with one of the amino acid sequences

VIR-121	LEAIPMSIPpEVAFNKPFVF
VIR-161	LEAIPCSIPpCVAFNKPFVF
VIR-162	LEAIPCSIPPCVGFGKPFVF
VIR-163	LEAIPCSIPPCVLFNKPFVF
VIR-164	LEAIPCSIPPCVFFNKPFVF
VIR-165	LEAIPCSIPPCFAFNKPFVF
VIR-166	LEAIPCSIPPCVA(D-Tic)NKP(D-Tic)FVF
VIR-170	LEAIPMSIPPEVFFGKPFVF
VIR-175	LEAIPMSIPPEFLFGKPFVF
VIR-182	LEAIPMSIPPELAFAKPFVF
VIR-184	LEAIPMSIPPEIAFNKPFVF
VIR-190	LEAIPMSIPpEVGFGKPFVF
VIR-191	LEAIPMSIPpEVLFGKPFVF
VIR-192	LEAIPMSIPpEVFFGKPFVF

VIR-193	LEAIPMSIPpEFAFNKPFVF
VIR-197	LEAIPMSIPpEVFFNKPFVF
VIR-199	LEAIPMSIPpEFLFNKPFVF
VIR-229	LEAIPISIPpEVAFNKPFVF
VIR-234	LEAIPMGIPpEVAFNKPFVF
VIR-243	LEAIPMSIPPEFAFNKDFVF
VIR-252	LEDIPMSIPpEVAFNKPFVF
VIR-255	LEKIPMSIPpEVAFNKPFVF
VIR-257	LEAIPMSIPpEV(Cyclohexylalanin)FNKPFVF
VIR-258	LEAIPMSIPpE(1-Naphthylalanin)AFNKPFVF
VIR-259	LEAIPMSIPpE(p-FluoroPhenylanin)AFNKPFVF
VIR-260	LEAIPMSIPpEV(4-Pyridylalanin)FNKPFVF
VIR-261	LEAIPMSIPpE(3,3-Diphenylalanin)AFNKPFVF
VIR-262	LEAIPMSIPpEV(D-Tic)FNKPFVF
VIR-263	LEAIPMSIPpEV(L-Tic)FNKPFVF
VIR-264	LEAIPMSIPpEV(3-Benzothienylalanin)FNKPFVF
VIR-265	LEAIPMSIPpEV(3-Thienylalanin)FNKPFVF
VIR-266	LEAIPMSIPpEVWFNKPFVF
VIR-268	LEAIPMSIPpEVAFNK(L-Tic)FVF
VIR-269	LEAIPMSIPpEVAFNK(Oic)FVF.

6. Nucleic acids coding for peptides according to any of claims 1 to 5.

7. Antibodies binding specifically to peptides according to claims 1 to 5.

8. A medicament containing the peptides according to claims 1 to 5, nucleic acids of claim 6 or antibodies of claim 7.

9. The medicament of claim 7 in galenic formulations for oral, intravenous, intramuscular, intracutaneous, subcutaneous, intrathecal administration, and as an aerosol for transpulmonary administration.

10. The medicament of claim 8 or 9 comprising at least one further therapeutic agent.

11. The medicament of claim 10, wherein the said at least one further therapeutic agent is a viral protease inhibitor, a reverse transcriptase inhibitor, a fusion inhibitor, a cytokine, a cytokine inhibitor, a glycosylation inhibitor or a viral mRNA inhibitor.

12. Use of peptides according to claims 1 to 5 for the manufacturing of a medicament for the treatment of HIV infections.

13. A diagnostic agent containing peptides according to any of claims 1 to 5, nucleic acids according to claim 6 or antibodies according to claim 7.

14. Use of the diagnostic agent according to claim 13 for assay systems for testing isolated plasma, tissue, urine and cerebrospinal fluid levels for HIV infections.

Summary:

The invention relates to peptides having the amino acid sequence

Z_1-LEAIP-X_3-SIP-X_1-X_4-V-X_2-FNKPFVF-Z_2,

wherein

X_1 is a D-proline or L-proline;

X_2 is an amino acid with a hydrophobic or an aromatic side chain or lysine;

X_3 and X_4 are cysteines, or X_3 is methionine and X_4 is glutamic acid;

Z_1 is NH_2 or a sequence of 1 to 10 amino acid residues;

Z_2 is COOH or a sequence of 1 to 10 amino acid residues;

their fragments and/or covalently linked oligomers and/or derivatives, especially amidated, acylated, sulfated, pegylated, phosphorylated and/or glycosylated derivatives, and mutants,

with biological activity against infection by HIV retroviruses,

with the proviso that at least one of the following is true:

X_1 is D-proline or

X_2 is not lysine or

X_3 and X_4 are cysteine,

and the use of such peptides in the treatment of infections by HIV.

Figure 1

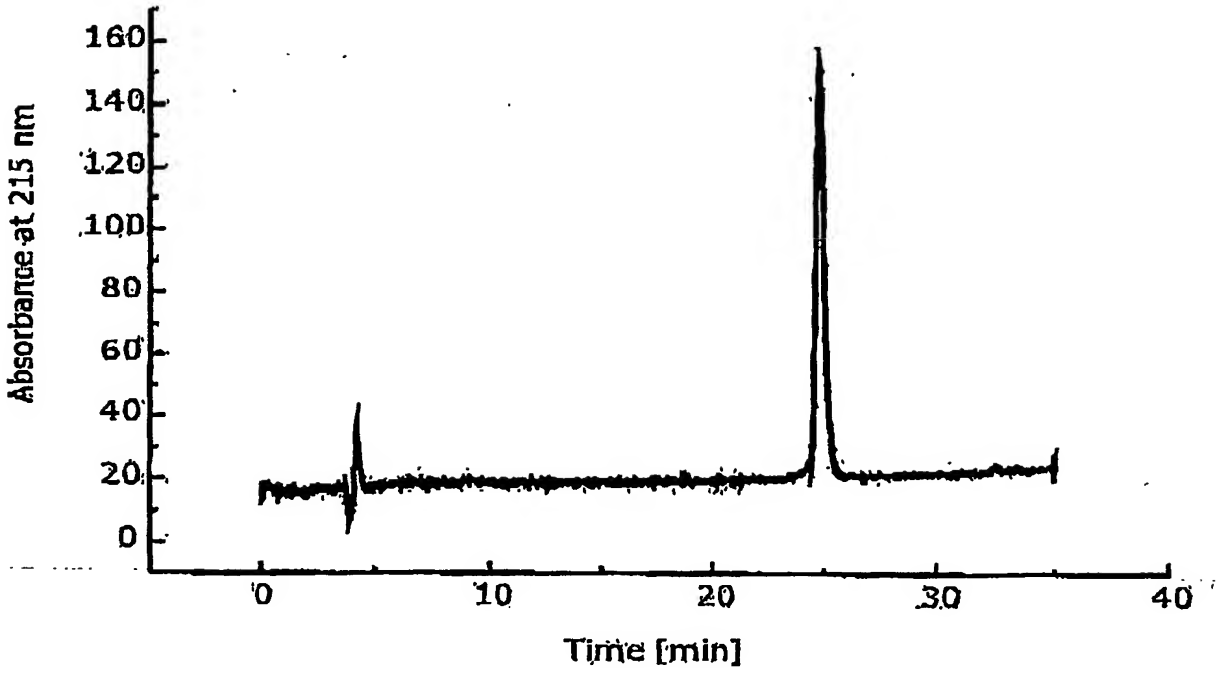
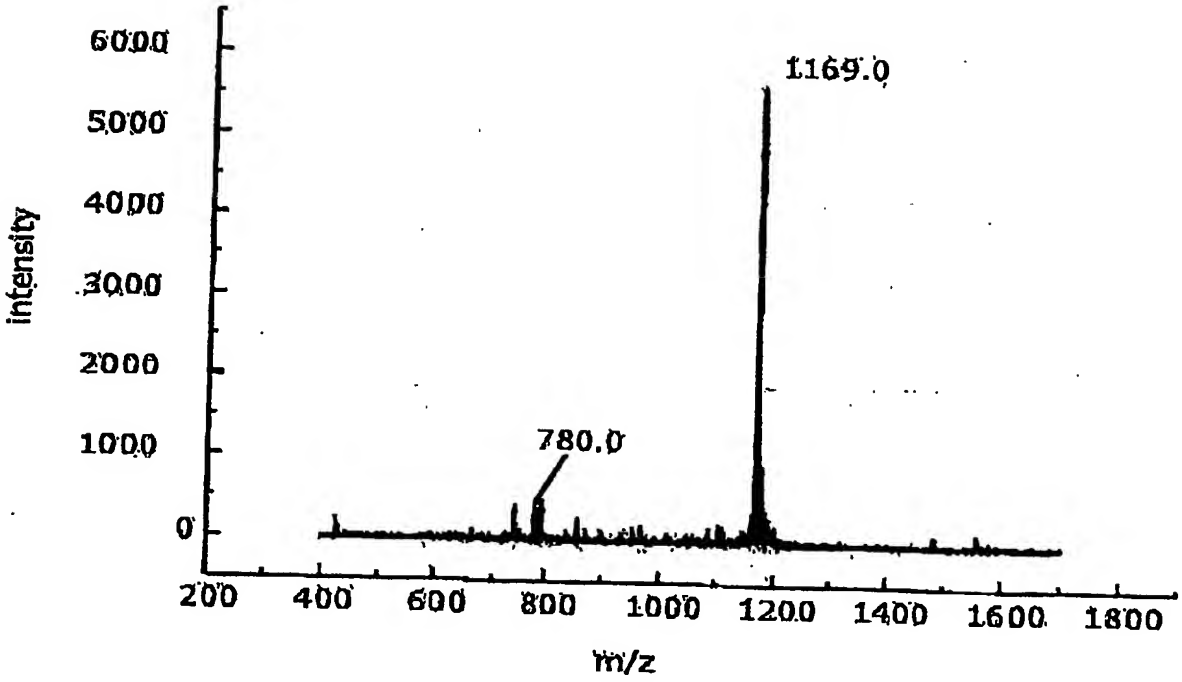

Absorbance at 215 nm
160
140
120
100
80
60
40
20
0
0
10
20
30
40
Time [min]

Figure 2


6000
5000
4000
3000
2000
1000
0
Intensity
1169.0
780.0
200
400
600
800
1000
1200
1400
1600
1800
m/z

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